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Gamma-Radiation Induced Apoptotic and Inflammatory Degeneration of Mouse Ovarian Follicles : Informative Biological-End Point for Disaster-Prevention

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Abstract

In mammals, most of the follicles can not be ovulated, and instead, are degenerated throughout the entire reproductive period. However, the precise mechanism of follicle atresia is unknown. Three weeks old female mice (ICR strain) were γ-irradiated with a dose of LD_{50}. Before irradiation (day 0) and at day 1, 2, and 3 after irradiation, the normal and atretic preantral and antral follicles of the left ovaries were morphologically observed. Atretic follicles at 2 days after irradiation had numerous cell debris, apoptotic cells and bodies, and polymorphonuclear leukocytes in the antral cavity. In severely atretic follicles, numerous polymorphonuclear leukocytes infiltrated into the follicle. The frequencies of atretic antral (58.0 $\pm$8.6) and preantral follicles (27.3$\pm$11.2) induced by γ-radiation increased to 94.0$\pm$3.4 and 86.9$\pm$7.6, respectively at 2 days after irradiation ($p<0.05$). The number of follicles with one or more neutrophils in the largest cross sections at 2 and 3 days after irradiation significantly increased ($p<0.05$). It can be concluded that γ-radiation triggers the recruitment of neutrophils into the follicles during degeneration. The ovarian follicles can make a role of informative biological end-point useful for disaster-prevention.

Key Words : radiation, ovarian follicle, apoptosis, neutorphil, disaster-prevention

1. Introduction

In mammals, most of the follicles can not be ovulated, and instead, are degenerated throughout the entire reproductive period. The degenerating follicles eventually disappeared in the ovary. It was reported that the degenerating follicles showed peculiar morphological characteristics such as an increase of pyknotic nuclei of granulosa cells, hypertrophed theca layer, and ruptured or

undulated basement membrane [1,2]. However, the precise mechanism of follicle atresia is unknown.

Ovarian follicular degeneration or atresia is a hormonally controlled apoptotic process, whereby degenerating follicles are eliminated in a coordinated fashion [3]. Apoptosis, a regulated form of cell death, is a physiological process essential for normal tissue homeostasis [4] in the absence of immune surveillance [5]. One of such pathologic stimuli that accelerate the follicle atresia was radiation [6]. In both normal tissues and tumors apoptosis occurs spontaneously and can be induced following irradiation [7]. Radiation induces cell apoptosis [7] and impairs the ovarian functions [8]. It was reported that the follicle atresia induced by radiation was mediated by apoptosis of granulosa cells in primordial and primary [9], preantral and antral follicles [10].

In human antral follicles, relatively large numbers of neutrophils were contained within the theca vasculature and the density of neutrophil was greater in atretic versus healthy follicles [11]. It suggested that there is a possibility that follicular atresia is mediated by apoptosis as well as the inflammatory immune response. Recently various biological end-points have been studied in relation to their informative role for risk assessment and disaster-prevention [12,13]. Since ovarian follicles are most sensitive to radiation, it can make a role of giving biological information necessary for the preventive measure in case of radiation disaster or nuclear accident. Therefore, the present study was carried out to investigate the morphological changes of follicular degeneration caused by radiation in the immature mouse ovary.

2. Materials and Methods

Three weeks old female mice (ICR strain) were obtained from Toxicology Research Center, Korea Research Institute of Chemical Technology, Taejon, Korea. The mice were raised in a 22℃ controlled animal care room with the light/dark cycle of 12/12h. The animals had free access to tap water and commercial chow during the experiments. Each experimental group consists of three mice.

Mice were irradiated with γ-radiation. The whole-body irradiation was carried out with a ^{60}Co isotopic source (dose rate: 12.0 cGy/min., source strength: approximately 150 TBq, Panoramic Irradiator, Atomic Energy of Canada Ltd.) at Korea Atomic Energy Research Institute (KAERI) as previously reported by Kim *et al.* [6]. The radiation dose was LD_{50} (7.2 Gy) and the irradiation time was 1 hour. Sham-irradiation of the control mice was performed by settlement of mice in the control room of irradiation facility during the radiation group was irradiated.

Before irradiation (day 0) and at day 1, 2, and 3 after irradiation, the left ovaries were dissected out and immediately fixed in 2.5% glutaraldehyde/0.1 M phosphate buffer (pH 7.3). Post fixation was followed using 1% of osmium tetraoxide (Sigma Chem. Co. MO) and was conducted for 2h at 4℃. Embedding of specimens after alcoholic dehydration and displacement by propylene oxide was carried out in epon mixture [Poly/Bed 812 resin (Epon 812): Dodecenylsuccinic Anhydride: Nadic Methyl Anhydride:2, 4, 6-tri (dimethylaminomethyl) phenol (DMP-30) = 19.3:12.3:9.4:0.6 ml, Polysciences Inc.]. Using ultramicrotome (Leica), semithin sections was prepared by 1μm in thickness and stained with 1% toluidine blue O in 1% borax solution. The largest cross sections were observed in this study. Observation of morphological changes was done under a light microscope (Olympus BX50) with the magnifications of ×400. Preantral and antral follicles in the largest cross-sections of the whole ovaries were observed. Follicles with one or more

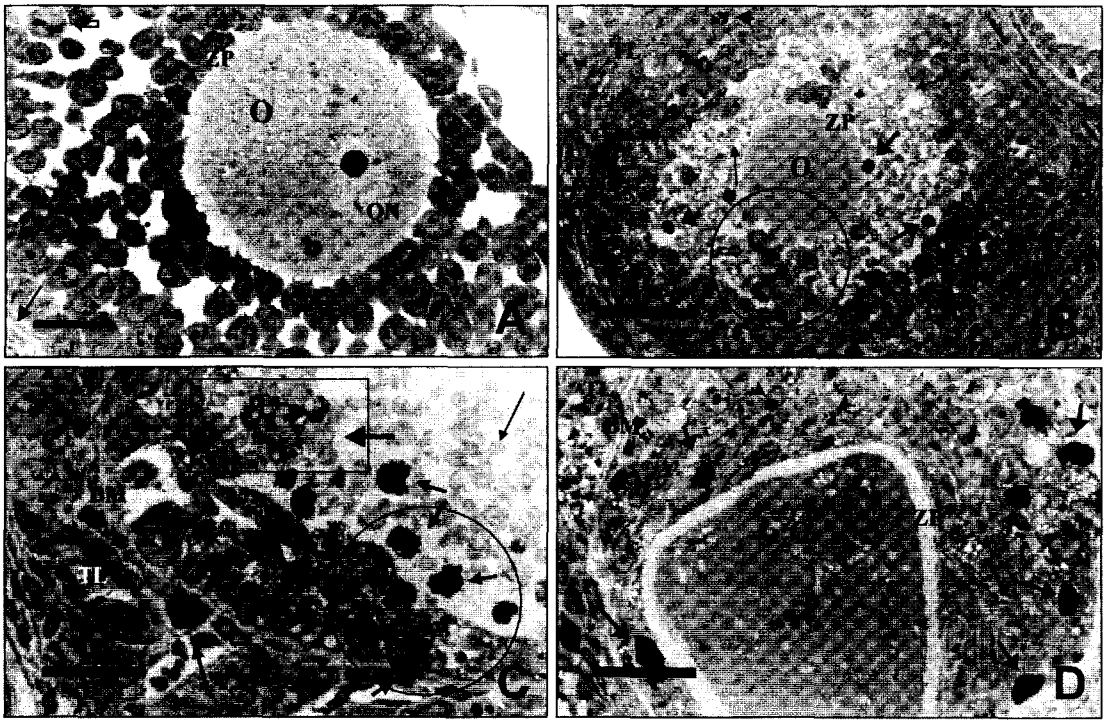

Fig. 1. Microphotographs of Ovarian Follicles in γ-irradiated Mice

A, normal oocyte with normal granulosa cells. Mitotic cells (open arrows) were shown. Thin arrows indicate basement membrane (bar, 20 ㎛); **B**, damaged follicle at 2 days post irradiation. Numerous cell debris (thin arrows) were shown in the follicle cavity. Apoptotic bodies (arrow heads) and polymorphonuclear leukocytes (thick arrows) were shown. One mitotic cell (open arrow) was observed (bar, 40 ㎛); **C**, atretic follicle at 1 day after irradiation. In a follicular cavity, numerous cell debris (thin arrow), polymorphonuclear leukocytes (arrows), and apoptotic bodies (arrow heads) were shown. In a box, one macrophage-like phagocytic cell (thick arrow) with apoptotic debris was observed. In a circle, one polymorphonuclear leukocyte infiltrated across the basement membrane (bar, 20 ㎛); and **D**, severely atretic preantral follicle 2 days after irradiation. Within the undulating basement membrane, numerous polymorphonuclear leukocytes (arrows) were observed. Zona pellucida and apoptotic bodies (arrow heads) were shown (bar, 20 ㎛). Abbreviations: **O**, oocyte; **ZP**, zona pellucida; **BM**, basement membrane; **TL**, theca layer, and **ON**, nucleus of oocyte.

mitotic granulosa cells and linear membrana granulosa, and without pyknotic granulosa cells as well as with linear and peculiar zona pellucida were classified into normal according to the criteria reported previously [10]. Follicles undefined by the above criteria were regarded as atretic. The largest cross sections of each preantral and antral follicle were identified by the comparison of the serial sections.

Under a light microscope, the number of normal and atretic follicles was counted. To compare the follicle status in ovary, the frequency (%) of normal follicles was calculated by the equation of [(normal follicles/total follicles) × 100]. All the data were expressed as mean ±SEM. The statistical differences were analyzed by Student's *t*-test and considered significant when p value was less than 0.05.

Table 1. Ratio of the Number of Follicles with Polymorphonuclear Leukocytes to that of Total Preantral and Antral Atretic Follicles

Group	Days after irradiation			
	0*	1	2	3
Follicles with neutrophil (%)	29.32±12.03**	25.48±7.50	65,91±11.49+	57.78±15.41+

* Data from the non-irradiated control group are expressed as day 0.

** Data are expressed as mean±SEM.

+ $p<0.05$ significantly different when compared to the value of day 0 of the control group.

3. Results

3.1. Morphology

Normal follicles contained mitotic granulosa cells in the membrana granulosa. The oocyte and zona pellucida had healthy appearances (Fig. 1A). Atretic follicles at 2 days after irradiation had numerous cell debris, apoptotic cells and bodies, and polymorphonuclear leukocytes in the antral cavity (Fig. 1B). Polymorphonuclear leukocytes infiltrated into the follicle cavity across the basement membrane. The atretic follicles also contained phagocytic cells (Fig. 1C). In severely atretic follicles, numerous polymorphonuclear leukocytes infiltrated into the follicle. Such a follicle had apoptotic cells and ruptured cells as well (Fig. 1D).

3.2. Atretic Frequency

The frequencies of atretic antal (Fig. 2) and preantral follicles (Fig. 2) induced by γ-radiation increased at 2 and 3 days after irradiation ($p<0.05$). In the control group before irradiation, the atretic frequencies were 58.0±8.6 and 27.3 ± 11.2 in antral and preantral follicles, respectively. At 1 day after irradiation, the frequency was not different from that of the control. However, at 2 days after irradiation, the frequencies increased to 94.0±3.4 and 86.9± 7.6 in antral and preantral follicles, respectively.

3.3. Neutrophil

The frequencies (%) of the number of follicles with one or more neutrophils to the entire number of follicles in the largest cross sections were 29.3 ±12.0 in the non-irradiation control mice (Table 1). The frequencies increased to 65.9±11.5 and 57.8±15.4 at 2 and 3 days after irradiation ($p<0.05$).

4. Discussion

In the previous study, we showed that γ-radiation induced the acute degeneration in mouse ovarian follicles [6]. It was proven that the follicular degeneration induced by radiation was mediated by apoptosis of granulosa cells. The criteria by which apoptosis is characterized include the loss of cell volume accompanied by nuclear pyknosis resulting from margination of the chromatin and its redistribution against the nuclear envelope [14]. There was a possibility that the immune system was damaged by high dose of radiation. In the present study, the mice were irradiated with LD_{50} for 1 hour. The results clearly showed that γ-radiation-

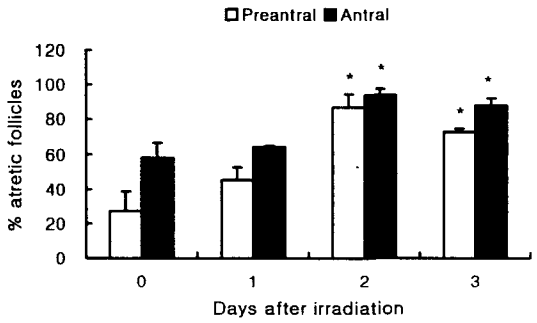

Fig. 2. Atretic Frequencies of Antral and Preantral Follicles in the Ovary Immature mice were γ-irradiated with a dose of 7.2Gy (LD_{50}) for 1 hour. Before irradiation and at 1, 2, and 3 days after irradiation, histological changes of left ovaries were observed. In the largest cross sections, the atretic follicles were counted under a microscope. Follicles with one or more apoptotic granulosa cells and bodies were classified into atretic ones. The atretic frequency was obtained by dividing the number with the total follicle number and presented as mean ±SEM. The number of mice a group was 3. *, $p<0.05$ significantly different from that of the control (day 0).

induced follicular degeneration was mediated by apoptosis of granulosa cells as well as by the inflammatory polymorphonuclear leukocytes (neutrophils). Chang *et al.* [11] reported that neutrophils were present in the theca of developing antral follicles, and increase in number during atresia in human follicles.

Best *et al.* [15] revealed the leukocyte subpopulations in human ovary. In the present experiment, it was identified that the number of infiltrated inflammatory neutrophils and apoptotic granulosa cells in the atretic follicles was increased at 2 and 3 days after irradiation. And the number of follicles with apoptotic cells and neutrophils significantly increased in the largest cross sections. It suggests that γ-radiation-induced follicular degeneration is mediated by the apoptosis of granulosa cells and by the inflammatory immune response.

The present study did not reveal the entrance of neutrophil into the follicle through the basement membrane. However, it is possible that neutrophils present in the follicle infiltrate into the follicles across the basement membrane. Typically, atretic follicles contained numerous cell debris and apoptotic granulosa cells or bodies in the follicle. Cell debris and apoptotic bodies were ingested by macrophage and neighboring phagocytic granulosa cells [16]. It can be thought that the inflammatory response mediated by neutrophils has the pivotal role in the elimination of damaged follicles in the ovary.

Since ovarian follicles are most sensitive to radiation, it can give biological information necessary for the preventive measure in case of radiation disaster or nuclear accident. The results of this study suggest that preantral and antral follicles can be used as an informative biological end-point for assessing the consequence of radiation disaster.

5. Conclusions

Gamma-radiation induces the degeneration of antral and preantral follicles in mouse ovary. The degenerated follicles contain the numerous apoptotic bodies and cells, cell debris, and polymorphonuclear leukocytes (neutrophils). The atretic frequency increases significantly at 2 days after irradiation. In concurrence with the incidence of follicle degeneration, neutrophils infiltrate into the follicle cavity. The present study shows that γ-radiation-induced follicular degeneration is mediated by apoptosis of granulosa cells as well as by the inflammatory immune response and that follicular degeneration is an informative phenomenon useful for assessing biological

consequence of radiation disaster. Although the precise roles of neutrophil during the atresia are not clearly defined, possible explanations that are amenable to further study do exist.

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